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RESEARCH HIGHLIGHT

HIF-1 α a novel piece in the NF- κ B puzzle

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Hypoxia, or low oxygen availability, is an important physiological stimulus for multicellular organisms. Molecularly, hypoxia induces a transcriptional programme directed at restoration of oxygen homeostasis and cellular survival. Hypoxia and inflammation are intricately linked, and even though it is appreciated that NF- κ B regulates the HIF system, little is known about how HIF regulates NF- κ B. In a recent report we have shown that HIF-1 α has an important role in regulating NF- κ B. Importantly, HIF-1 α acted to constrain NF- κ B transcriptional activity, in mammalian cells and in the *in vivo* genetic model of *Drosophila*. Reduction of HIF-1 α resulted in increased levels of specific NF- κ B targets, by a mechanism dependent on the TAK/IKK and CDK6 kinases. Deletion of the HIF-1 α homologue in *Drosophila*, Sima, resulted in heightened sensitivity to infection due to uncontrolled NF- κ B. This report delineated for the first time the contribution of HIF-1 α towards the NF- κ B pathway, and demonstrated the importance of HIF-1 α presence for the restraint of the inflammatory response *in vivo*. The importance of this crosstalk between HIF and NF- κ B is significant, as it could create potential new therapies in diseases where hypoxia and inflammatory are prevalent.

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Oxygen homeostasis is crucially important for the survival of all vertebrate species^[1]. Therefore, organisms developed a way to coordinate the oxygen supply in order to maintain the balance that the cells need to function properly. Cells have evolved mechanisms to control oxygen levels in the intracellular compartments. When these mechanisms fail, and the standards of oxygen supply decrease, a stress condition called hypoxia is created. Hypoxia is also a relevant physiological stress associated with many processes such as adaptation to high altitudes and embryo development but also involved in many pathological conditions such as cancer, heart failure and stroke^[2, 3]. An essential component responsible to regulate the molecular response to hypoxia in the cell is the transcription factor family called Hypoxia Inducible Factor (HIF). HIF is a complex of transcription factors composed of an α - and a β - subunit. While the α -subunit changes with oxygen levels, the β - does not, and it is

constitutively expressed. The oxygen-dependent control of HIF- α is conferred by a class of dioxygenases called prolyl-hydroxylases (PHDs), whose catalytic activity marks HIF- α for VHL-dependent proteasomal degradation when oxygen is present^[4]. The hypoxia-responsive HIF complex is responsible for the initiation of a transcriptional program to hypoxia, and ultimately restoration of oxygen homeostasis^[5, 6].

Another important stress of medical relevance is Inflammation. Inflammation is a protective attempt to eliminate pathogens but is also involved in the initiation of the healing process of a wound. Similarly to hypoxia, cells have evolved sophisticated mechanisms to control the inflammatory response to pathogens^[7]. A key element of these mechanisms is a family of transcription factors called Nuclear Factor κ -light-chain-enhancer of activated B cells

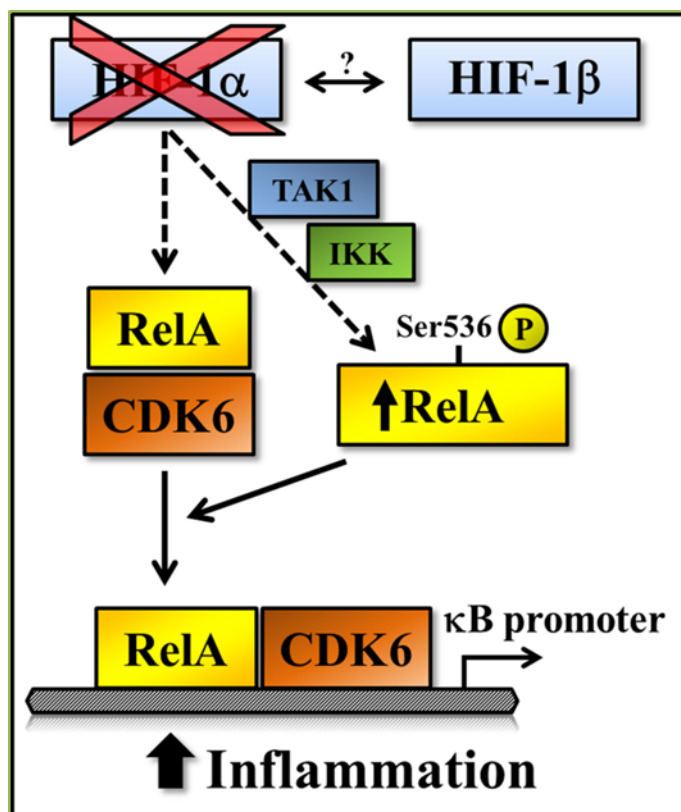


Figure 1: Model of HIF regulation over NF-κB pathway.

Depletion of HIF-1 α led to increase of RelA/CDK6 complex activity, increasing the expression of pro-inflammatory cytokines, such as IL-8. Additionally, lack of HIF-1 α results in increased RelA S536 phosphorylation, which can also lead to increase of expression of inflammatory signals. Whether other HIF subunits, such as HIF-1 β , or other NF- κ B subunits are involved remains unknown.

(NF- κ B). In mammals, NF- κ B is composed of five genes, encoding RelA/p65, RelB, cRel, p100/p52 and p105/p50^[8]. NF- κ B targets are extensive and include genes that control many different cellular processes such as proliferation, apoptosis, inflammation, migration and cell cycle^[8, 9]. This family of transcription factors are activated under several different stimuli (such as virus, bacteria or cytokines), activating several signalling pathways that ultimately engage a complex transcriptional program. Importantly, several diseases (e.g. cancer and rheumatoid arthritis), result from the deregulation of these pathways^[10-12].

Hypoxia and inflammation are intimately connected at the cellular, clinical and also at the molecular level^[2, 13]. HIF (hypoxia) and NF- κ B (inflammation) have previously been linked at the molecular level^[14-16]. On one hand, they share a number of common target genes. On the other hand, physical and functional interactions between HIF subunits and NF- κ B have been described^[13].

Our laboratory, and others, have previously demonstrated

that inflammatory stimuli, such as TNF- α , and bacterial products including LPS, led to HIF-1 α activation in a NF- κ B dependent manner in cancer cell lines^[14, 17]. These findings demonstrated NF- κ B as a pivotal regulator of HIF activity and expression. However, whether HIF had any role in the regulation of the NF- κ B pathway had not been elucidated, until very recently. Our study, recently published, has demonstrated the involvement of HIF in the regulation of NF- κ B^[18]. Using several mammalian cancer cell lines we showed that in the absence of HIF-1 α , NF- κ B activity was enhanced. Furthermore, in response to TNF- α several NF- κ B target genes, such as Cyld, A20, and I κ B- α , were up-regulated upon HIF-1 α siRNA knockdown. These results are in line with the idea of HIF having a protective role against inflammation. In this case, HIF would work as one of the negative feedback loops, so important to restrain NF- κ B activity, and mediate the response to infection^[18].

Mechanistically, in our study, we demonstrated that the increase in NF- κ B activity after HIF depletion required TAK, IKK and, at least in part CDK6 kinases^[18]. While IKK and TAK1 are kinases required for proper TNF- α and hypoxia mediated activation of NF- κ B^[8], CDK6 was previously described as a co-activator of NF- κ B target genes^[19, 20]. Our analysis revealed that CDK6 was not only required for the HIF-1 α mediated-repression of NF- κ B, but also for the phosphorylation of RelA at the Serine 536 in basal conditions (**Figure 1**). This phosphorylation is particularly important, as previous work has described to be important in the activation of NF- κ B target genes^[21]. Furthermore, CDK6 interaction with RelA at κ B promoters had been previously shown to be important for NF- κ B-dependent expression of specific genes^[19, 20]. Interestingly, we also showed that in the absence of HIF-1 α this interaction was enhanced, and consequently more RelA-CDK6 was found at the κ B promoter of the important chemokine IL-8^[18] (**Figure 1**).

Our findings were also extended to an *in vivo* model, using *Drosophila melanogaster*. *Drosophila* has been instrumental to determine the role of NF- κ B in infection and inflammation^[22]. The NF- κ B pathway is well conserved and importantly, so is the HIF pathway^[23]. Deletion of the HIF- α homologue in *Drosophila*, Sima, resulted in hypersensitivity to infection due to deregulated NF- κ B^[18]. Previous work from the laboratory had shown that *Drosophila* HIF- α was activated by inflammation *in vivo* in an IKK-dependent manner^[16], recapitulating the finding from cell culture work^[14, 24], however HIF's role in such circumstances had not been investigated. In our most recent study, we demonstrated that *Drosophila* HIF- α mutant flies had increased levels of the NF- κ B subunits, and targets genes, which resulted in

deregulated immune response, and poor survival in response to infection^[18]. Interestingly, our results suggested that HIF was acting mainly through the IMD pathway, which has been strongly associated to the inflammatory production of antibacterial peptides and mediation of the inflammatory response in this model organism^[25].

So far very few reports have linked HIF with inflammation *in vivo*. In zebrafish, it was shown that HIF induction following inflammation resulted in decreased neutrophil apoptosis, and consequently in a delayed resolution phase of inflammation clearance^[26]. On the other hand, in mice, HIF has been described as a protective factor against inflammation in a colitis model, where mice lacking HIF-1 α in the intestinal epithelium correlated with more severe inflammatory response and mortality^[27]. Additional reports in mice have also shown HIF protective role in the intestine^[28, 29].

Taking in consideration our findings, and the reports on HIF's role in inflammation, it seems that, depending on cell type, HIF-1 α can be either pro-inflammatory or anti-inflammatory. Even though, it seems clear that HIF controls the inflammatory response through the regulation of NF- κ B, what determines if it acts as an activator or a repressor is not. Therefore, it would be interesting to investigate the contribution of each HIF and NF- κ B subunit in the context of inflammation. This analysis should also be extended to different cell types such as the immune system cell subtypes as well as fibroblasts and additional cancer cell models. This would be important not only to understand HIF's antagonistic effects on NF- κ B, but also to create potential new therapies in inflammatory diseases and cancer.

In conclusion, our study revealed another piece in the puzzle that is NF- κ B regulation, in the form of HIF-1 α . HIF-1 α presents itself as a novel negative regulator of exacerbated NF- κ B activity, for which it modulates the activity of TAK-IKK axis as well as CDK6 (**Figure 1**). However, whether HIF-1 α requires its binding partner, HIF-1 β , for this role is currently unclear and future work should reveal if this is the case. It does, however, raise the interesting possibility of elevating HIF-1 α as a potential therapeutic in inflammatory diseases. This should be a feasible and testable possibility, as several PHD inhibitors, known to activate HIF-1 α levels and activity, are currently in clinical trials for conditions of anaemia^[30].

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